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- [54] DNA ENCODING FOR THE PRECURSOR PROTEIN OF APC POLYPEPTIDE ASSOCIATED WITH ALZHEIMER'S DISEASE
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- [52] U.S. Cl. .... 536/23.5; 530/350; 530/388.1
- [58] Field of Search ..... 536/27, 28, 29

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### [57] ABSTRACT

The present invention relates to the precursor protein of amyloid plaque core (APC) polypeptide, to fragments of the precursor protein and to the diagnostic use of the precursor protein and of the fragments. Furthermore, the invention relates to the DNA coding for the precursor protein, to fragments of this DNA and to the diagnostic use of the DNA and of the fragments.

3 Claims, 3 Drawing Sheets

AGTTTCCTCGGCAGCGGTAGGCGAGA -121  
GCACGCGGAGGAGCGTGCGCGGGGCCCCGGGAGACGGCGGGCGGTGGCGGGCGGGGCAGAG -61  
CAAGGACGCGGGCGGATCCCCTCGCACAGCAGCGCACTCGGTGCCCGCGCAGGGTCGCG -1  
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCTGGACGGCTCGGGCGCTGGAGGTA 60  
M L P G L A L L L L A A W T A R A L E U  
1 10 20  
CCCCTGATGGTAATGCTGGCCTGCTGGCTGAACCCAGATTGCCATGTTCTGTGGCAGA 120  
P T D G N A G L L A E P O I A M F C G R  
30 40  
CTGAACATGCACATGAATGTCCAGAATGGGAAGTGGGATTCAGATCCATCAGGGACCAAA 180  
L N M H M N U Q N G K W D S D P S G T K  
50 60  
ACCTGCATTGATACCAAGGAAGGCATCCTGCAGTATTGCCAAGAAGTCTACCCTGAACTG 240  
T C I D T K E G I L O Y C O E U Y P E L  
70 80  
CAGATACCAATGTGGTAGAAGCCAACCAACCAGTGACCATCCAGAAGTGGTGCAAGCGG 300  
O I T N U U E A N O P U T I Q N W C K R  
90 100  
GGCCGCAAGCAGTGCAAGACCCATCCCCTTTGTGATTCCCTACCGCTGCTTAGTTGGT 360  
G R K Q C K T H P H F U I P Y R C L U G  
110 120  
GAGTTTGTAAGTGATGCCCTTCTCGTTCCTGACAAGTGCAAATTCTTACACCAGGAGAGG 420  
E F U S D A L L U P D K C K F L H Q E R  
130 140  
ATGGATGTTTGCGAAACTCATCTTCACTGGCACACCGTCGCCAAAGAGACATGCAGTGAG 480  
M D U C E T H L H W H T U A K E T C S E  
150 160  
AAGAGTACCAACTTGCATGACTACGGCATGTTGCTGCCCTGCCGAATTGACAAGTTCCGA 540  
K S T N L H D Y G H L L P C G I D K F R  
170 180  
GGGGTAGAGTTTGTGTGTTGCCCACTGGCTGAAGAAAGTGACAATGTGGATTCTGCTGAT 600  
G U E F U C C P L A E E S D N U D S A D  
190 200  
GCGGAGGAGGATGACTCGGATGTCTGGTGGGGCGGAGCAGACACAGACTATGCAGATGGG 660  
A E E D D S D U W W G G A D T D Y A D G  
210 220  
AGTGAAGACAAAGTAGTAGAAGTAGCAGAGGAGGAAGAAGTGGCTGAGGTGGAAGAAGAA 720  
S E D K U U E U A E E E E U A E U E E E  
230 240  
GAAGCCGATGATGACGAGGACGATGAGGATGGTGTGAGGTAGAGGAAGAGGCTGAGGAA 780  
E A D D D E D D E D G D E U E E E A E E  
250 260  
CCCTACGAAGAAGCCACAGAGAGAACCACCAGCATTGCCACCACCACCACCACCACCACA 840  
P Y E E A T E R T T S I A T T T T T T T  
270 280  
GAGTCTGTGGAAGAGGTGGTTCGAGTTCCTACAACAGCAGCCAGTACCCCTGATGCCGTT 900  
E S U E E U U R U P T T A A S T P D A U  
290 300  
GACAAGTATCTCGAGACACCTGGGGATGAGAATGAACATGCCCATTTCCAGAAAGCCAAA 960  
D K Y L E T P G D E N E H A H F Q K A K  
310 320

FIG.1a



GAGAGGCTTGAGGCCAAGCACCGAGAGAGAATGTCCCAGGTCATGAGAGAATGGGAAGAG 1020  
E R L E A K H R E R M S Q V M R E W E E  
330 340

GCAGAACGTCAAGCAAAGAACTTGCCTAAAGCTGATAAGAAGGCAGTTATCCAGCATTTC 1080  
A E R Q A K N L P K A D K K A V I O H F  
350 360

CAGGAGAAAGTGGAAATCTTTGGAACAGGAAGCAGCCAACGAGAGACAGCAGCTGGTGGAG 1140  
Q E K V E S L E Q E A A N E R Q O L V E  
370 380

ACACACATGGCCAGAGTGGAAAGCCATGCTCAATGACCGCCGCCGCCTGGCCCTGGAGAAC 1200  
T H M A R V E A M L N D R R R L A L E N  
390 400

TACATCACCGCTCTGCAGGCTGTTCTCCTCGGCCTCGTCACGTGTTCAATATGCTAAAG 1260  
Y I T A L Q A V P P R P R H U F N M L K  
410 420

AAGTATGTCCGCGCAGAACAGAAGGACAGACAGCACACCCTAAAGCATTTCGAGCATGTG 1320  
K Y U R A E O K D R O H T L K H F E H U  
430 440

CGCATGGTGGATCCCAAGAAAGCCGCTCAGATCCGGTCCCAGGTTATGACACACCTCCGT 1380  
R M V D P K K A A Q I R S Q U M T H L R  
450 460

GTGATTTATGAGCGCATGAATCAGTCTCTCTCCCTGCTCTACAACGTGCCTGCAGTGGCC 1440  
V I Y E R M N Q S L S L L Y N U P A U A  
470 480

GAGGAGATTCAGGATGAAGTTGATGAGCTGCTTCAGAAAGAGCAAAACTATTCAGATGAC 1500  
E E I Q D E V D E L L Q K E Q N Y S D D  
490 500

GTCTTGGCCAACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCTCATGCCA 1560  
V L A N M I S E P R I S Y G N D A L M P  
510 520

TCTTTGACCGAAACGAAAACCACCGTGGAGCTCCTTCCCGTGAATGGAGAGTTCAGCCTG 1620  
S L T E T K T T V E L L P U N G E F S L  
530 540

GACGATCTCCAGCCGTGGCATTCTTTTGGGGCTGACTCTGTGCCAGCCAACACAGAAAAC 1680  
D D L Q P W H S F G A D S U P A N T E N  
550 560

GAAGTTGAGCCTGTTGATGCCCGCCCTGCTGCCGACCGAGGACTGACCACTCGACCAGGT 1740  
E V E P V D A R P A A D R G L T T R P G  
570 580

FIG. 1b

TCTGGGTTGACAAATATCAAGACGGAGGAGATCTCTGAAGTGAAGATGGATGCAGAATTC 1800  
 S G L T N I K T E E I S E U K M D A E F  
 590 600

CGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCCTTGCAGAAGATGTG 1860  
 R H D S G Y E U H H Q K L U F F A E D U  
 610 620

GGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGACAGTG 1920  
G S N K G A I I G L M U G G U U I A T U  
 630 640

ATCGTCATCACCTTGGTGTGCTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTG 1980  
I U I T L U M L K K K Q Y T S I H H G U  
 650 660

GTGGAGGTTGACGCCGCTGTCACCCAGAGGAGCGCCACCTGTCCAAGATGCAGCAGAAC 2040  
 U E U D A A U T P E E R H L S K M Q Q N  
 670 680

GGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAACTAGACCCCGCCACA 2100  
 G Y E N P T Y K F F E Q M Q N \*

GCAGCCTCTGAAGTTGGACAGCAAACCATGCTTCACTACCCATCGGTGTCCATTTATA 2160

GAATAATGTGGGAAGAAACAAACCCGTTTTATGATTTACTCATTATCGCCTTTTGACAGC 2220

TGTGCTGTAACACAAGTAGATGCCTGAACTTGAATTAATCCACACATCAGTAATGTATTC 2280

TATCTCTCTTTACATTTTGGTCTCTATACTACATTATTAATGGGTTTTGTGTAAGTAAA 2340

GAATTTAGCTGTATCAAACCTAGTGCATGAATAGATTCTCTCCTGATTATTTATCACATAG 2400

CCCCTTAGCCAGTTGTATATTATTCTTGTGGTTTGTGACCCAATTAAGTCCTACTTTACA 2460

TATGCTTTAAGAATCGATGGGGGATGCTTCATGTGAACGTGGGAGTTCAGCTGCTTCTCT 2520

TGCCTAAGTATTCCTTTCCTGATCACTATGCATTTTAAAGTTAAACATTTTAAAGTATTT 2580

CAGATGCTTTAGAGAGATTTTTTTTCCATGACTGCATTTTACTGTACAGATTGCTGCTTC 2640

TGCTATATTTGTGATATAGGAATTAAGAGGATACACACGTTTGTTCCTTCGTGCCTGTTT 2700

TATGTGCACACATTAGGCATTGAGACTTCAAGCTTTTCTTTTTTTGTCCACGTATCTTTG 2760

GGTCTTTGATAAAGAAAAGAATCCCTGTTCAATTGTAAGCACTTTTACGGGGCGGGTGGGG 2820

AGGGGTGCTCTGCTGGTCTTCAATTACCAAGAATTCTCCAAAACAATTTTCTGCAGGATG 2880

ATTGTACAGAATCATTGCTTATGACATGATCGCTTTCTACACTGTATTACATAAATAAAT 2940  
TAAATAAATA

TAAATAAATAACCCCGGGCAAGACTTTTCTTTGAAGGATGACTACAGACATTAATAAAT 3000

CGAAGTAATTTTGGGTGGGGAGAAGAGGCAGATTCAATTTTCTTTAACCAGTCTGAAGTT 3060

TCATTTATGATACAAAAGAAGATGAAAATGGAAGTGGCAATATAAGGGGATGAGGAAGGC 3120

ATGCCTGGACAAACCTTCTTTTAAGATGTGTCTTCAATTTGTATAAAATGGTGTTTTCA 3180

TGTAATAAATATACATTCTTGGAGGAGC - poly (A) tail

FIG. 1c



## DNA ENCODING FOR THE PRECURSOR PROTEIN OF APC POLYPEPTIDE ASSOCIATED WITH ALZHEIMER'S DISEASE

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The present invention relates to the precursor protein of amyloid plaque core (APC) polypeptide, to fragments of the precursor protein and to the diagnostic use of the precursor protein and of the fragments. Furthermore, the invention relates to the DNA coding for the precursor protein, to fragments of this DNA and to the diagnostic use of the DNA and of the fragments.

Alzheimer's disease was described as an independent clinical and pathological entity for the first time in the year 1907 by the German neurologist Alois Alzheimer (Alzheimer, A. (1907) *Zentralblatt für Nervenheilkunde und Psychiatrie*, 177-179). It is the commonest degenerative brain disease of old people. In America alone about 2 million people are now suffering from the disease, and at least 100,000 die of it each year (Wurtman, R. J. (1985) *Sci. Am.* 252, 48-56).

The disease appears in people between 40 and 80 years of age. Those affected gradually lose their memory and their ability to concentrate. The state of mental deterioration advances until, within 3 to 10 years, the patients are unable either to speak, to think or to take care of themselves, and finally they die. The cause of this dementia is unknown. There is neither a definitive diagnosis nor a therapy.

Brain autopsies of people who have died of Alzheimer's disease reveal typical changes under the microscope as follows:

There has been a decrease in the number of neurons, especially in the parietal lobes, that is to say in the parts of the brain where the memory functions are localized. A loss of neurons which normally release acetylcholine is likewise clearly visible.

In addition, three extremely unusual structures appear in the cerebral cortex, these structures not existing in the brain of healthy people and thus being used for diagnosis (after death):

- 1) intracellular neurofibrils  
(NFTs, neurofibrillary tangles)

In the cytosome of neurons of the cerebral cortex and of the hippocampus are found bundles consisting of two filaments which are twisted around one another in the manner of a helix (PHFs, paired helical filaments).

- 2) extracellular amyloid plaques  
(APC, amyloid plaque core)

The neuritic plaques contain amyloid and the residues of dead cells, and they are scattered over the cerebral cortex, the hippocampus and the amygdaloid nucleus. The number of plaques is correlated with the degree of mental deterioration.

- 3) cerebrovascular amyloid  
(ACA, amyloid congophilic angiopathy)

Amyloid is the name given to a protein-rich composition. Such amorphous protein aggregates are to be found all around the blood vessels and in the wall of blood vessels in the brain.

The protein component of ACA has been isolated and sequenced (Glennner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* 120, 885-890). The amino acid sequence has no homology with known protein sequences. The protein components of PHFs and APC have likewise been isolated and sequenced

(Masters, C. L., Multhaupt, G., Simms, G., Pottgiesser, J., Martins R. N. and Beyreuther, K. (1985) *EMBO* 4, 2757-2763 and Masters, C. L., Simms, G., Weinman, N. A., Multhaupt, G., McDonald, B. L. and Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* 82, 4245-4249). The amino acid sequences indicate that all three polypeptides are probably the same one having a molecular weight of 4.5 kD. The relevant sequence is shown in boxes in FIGS. 1a-c (positions 597-638).

There are several hypotheses to explain the origin of this APC protein. It might be a normal protein in the brain (or even in another organ) in which either regulation of biosynthesis has become deranged or physiological breakdown is impaired. The accumulations of very large amounts might then be the cause of the disease. If it is an abnormal protein, and its unusual ability to aggregate causes the disease, it might also be coded for by a healthy human gene which was under faulty control due to some factor or other, for example, viruses, foodstuffs or environmental toxins. The fault might also comprise a modification of the original protein precursor. On the other hand however, a viral gene might also be responsible for synthesizing the APC protein.

In the work leading to the invention an attempt has now been made to establish the origin and nature of the APC protein, whose aggregation in the cerebral cortex is one of the main biochemical signs in Alzheimer patients, in order thereby to obtain a tool for improved diagnosis of Alzheimer's disease.

For this purpose, a human fetal brain c-DNA bank with pA+mRNA of the cerebral cortex was constructed.

The c-DNA was synthesized by the method of Okayama and Berg (Okayama, H. and Berg, P. *Mol. Cell. Biol.* 2, 161-170 (1982); Okayama, H. and Berg, P. *Mol. Cell. Biol.* 3, 280-289 (1983)), and the c-DNA was transformed into *E. coli* HB 101 (Aviv, H. and Leder, P. *Proc. Natl. Acad. Sci. USA* 69, 1408 (1972)). Each of the c-DNA banks obtained in this way contains more than  $1 \times 10^6$  independent c-DNA clones.

To screen the bank, use was made of a DNA probe whose sequence was derived from the sequence of APC polypeptide. The chosen sequence corresponds to the amino acids in positions 10-16 of APC. The relevant sequence is indicated by a brace in FIG. 1c (positions 1815-1835). In order to ensure optimum hybridization, the degeneracy of the genetic code was taken into account, and a mixture having the following sequence



was prepared and used as probe. This is a 64-fold degenerate 20-mer. A test on 100,000 c-DNA clones from the human fetal cerebral cortex bank resulted in the isolation of a complete (full-length) c-DNA clone, having the serial No. EC 9.110, which codes for a protein which contains the APC sequence and thus represents the precursor protein of APC peptide. The sequence of the c-DNA, and the amino acid sequence of the coded protein, are to be found in FIG. 1. Sequence analysis was carried out by the dideoxy method (Sanger, F., Nicklen, S. and Coulson, A. R. *Proc. Natl. Acad. Sci. USA* 74, 5463-5467 (1977) and Guidelines for quick and simple Plasmid Sequencing, Handbook, (1986) Boehringer Mannheim GmbH, Biochemica, D-6800 Mann-



heim). Nothing is known at present about the natural function of the APC precursor protein.

### SUMMARY OF THE INVENTION

Thus the present invention relates to the deoxyribonucleic acid of the sequence shown in FIG. 1 and to its functional equivalents. In this context, the term functional equivalents means that, owing to the degeneracy of the genetic code, individual nucleotides in the sequence can be exchanged or derivatized without this having an effect on the function of the nucleic acid. In particular, the invention relates to the DNA of the sequence shown in FIG. 1 from position 1 to position 2089, and to its functional equivalents. This part of the DNA is the part which codes for the precursor protein. Due to some peculiarities in the sequence, the protein and the corresponding DNA sequence are an interesting tool for the diagnosis of Alzheimer's disease at the molecular level. In this connection, the region from approximately position 600 to approximately position 900 is particularly worthy of mention. This part codes for a number of acidic amino acids which is unusually large in relation to the length of this section. Also worthy of very particular note are the seven consecutive threonines (position: DNA 819-840/amino acids 274-280). Such regions are particularly interesting for the development of DNA probes for diagnosis because, due to their unusual sequence, they are unique and thus allow highly specific detection.

The invention also relates to fragments of the DNA from FIG. 1 and to oligonucleotides derived from this DNA, and to their use as probes in diagnosis. The DNA is not used in its full length for hybridization experiments. Normally, fragments of a length of about 10 to 50 nucleotides are used for hybridizations. Longer fragments usually give rise to manipulation problems. Fragments with fewer than 10 nucleotides usually do not have adequate specificity, or the binding is too weak.

### DETAILED DESCRIPTION OF THE INVENTION

The DNA shown in FIG. 1, and the fragments of this DNA, can be used very satisfactorily for the diagnosis of Alzheimer's disease, to detect mutations such as, for example, deletions, insertions and point mutations or rearrangement errors.

The present invention makes it possible to diagnose Alzheimer's disease on the molecular level. This applies equally to the presymptomatic diagnosis of Alzheimer's disease. The analyses can be carried out with known techniques of DNA technology, such as, for example, the techniques described by Antonarkais et al. (1985) in Hum. Gen 69, 1-14.

The present invention also includes the precursor protein coded for by the DNA, and the fragments of this protein. The detection of this protein or of the fragments likewise represents an approach to the diagnosis of Alzheimer's disease. Once again, the peculiarities of the sequence (amino acids: about position 200 to about position 290) are of particular importance. Fragments of the precursor protein, especially from the region 200 to 290, can be used very satisfactorily as antigens peptides for the preparation of polyclonal or monoclonal antibodies which, in turn, are used in diagnosis.

Functional equivalents in the context of the protein or the peptides means that variations, in the form of exchange of amino acids or derivatizations which have

no effect on the function of these peptides, for example as antigens, are possible both in the sequence of the protein and in the peptides too.

Key to FIGS. 1 a-c.

Nucleotide sequence 5'→3' of the c-DNA clone which codes for the precursor protein of APC polypeptide, and the amino acid sequence derived from the DNA. The amino acids are designated using the following one-letter code:

Amino acids	
A Ala	Alanine
B Asx	AsN or Asp
C Cys	Cysteine (cystine)
D Asp	Aspartic acid
E Glu	Glutamic acid
F Phe	Phenylalanine
G Gly	Glycerine
H His	Histidine
HS	Homoserine
HSL	Homoserine lactone
I Ile	Isoleucine
K Lys	Lysine
L Leu	Leucine
M Met	Methionine
N AsN	Asparagine
Nle	Norleucine
P Pro	Proline
Q Gln	Glutamine
R Arg	Arginine
S Ser	Serine
T Thr	Threonine
V Val	Valine
W TrP	Tryptophan
Y Tyr	Tyrosine
Z Glx	Glu or GIN
X	not identified

It will be appreciated that the instant specification and claims are set forth by way of illustration and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

What is claimed is:

1. A deoxyribonucleic acid of the sequence:

AGTTTCCTCGGCAGCGGTAGGCGAGA	-121
GCACGCGGAGGAGCGTGCGCGGGGCCCGG	-91
GAGACGGCGGCGGTGGCGGCGCGGGCAGAG	-61
CAAGGACGCGGCGGATCCCACTCGCACAGC	-31
AGCGCACTCGGTGCCCGCGCAGGGTCGCG	-1
ATGCTGCCCGGTTTGGCACTGCTCCTG CTG	30
M L P G L A L L L L	
GCCGCCTGGACGGCTCGGGCGCTGGAGGTA	60
A A W T A R A L E V	
CCCACTGATGGTAATGCTGGCCTGCTGGCT	90
P T D G N A G L L A	
GAACCCAGATTGCCATGTTCTGTGGCAGA	120
E P Q I A M F C G R	
CTGAACATGCACATGAATGTCCAGAATGGG	150
L N M H M N V Q N G	
AAGTGGGATTCAGATCCATCAGGGACCAAA	180
K W D S D P S G T K	
ACCTGCATTGATACCAAGGAAGGCATCCTG	210
T C I D T K E G I L	



-continued

CAGTATTGCCAAGAAGTCTACCCTGAACTG  
 Q Y C Q E V Y P E L  
 CAGATCACCAATGTGGTAGAAGCCAACCAA  
 Q I T N V V E A N Q  
 CCAGTGACCATCCAGAACTGGTGCAAGCGG  
 P V T I Q N W C K R  
 GGCCGCAAGCAGTGCAAGACCCATCCCCAC  
 G R K Q C K T H P H  
 TTTGTGATTCCCTACCGCTGCTTAGTTGGT  
 F V I P Y R C L V G  
 GAGTTTGTAAAGTGATGCCCTTCTCGTTCT  
 E F V S D A L L V P  
 GACAAGTGCAAATTCTTACACCAGGAGAGG  
 D K C K F L H Q E R  
 ATGGATGTTTGCGAAACTCATCTTCACTGG  
 M D V C E T H L H W  
 CACACCGTCGCCAAAGAGACATGCAGTGAG  
 H T V A K E T C S E  
 AAGAGTACCAACTTGCATGACTACGGCATG  
 K S T N L H D Y G M  
 TTGCTGCCCTGCGGAATTGACAAGTTCCGA  
 L L P C G I D K F R  
 GGGGTAGAGTTTGTGTGTTGCCCACTGGCT  
 G V E F V C C P L A  
 GAAGAAAGTGACAATGTGGATTCTGCTGAT  
 E E S D N V D S A D  
 GCGGAGGAGGATGACTCGGATGTCTGGTGG  
 A E E D D S D V W W  
 GGCGGAGCAGACACAGACTATGCAGATGGG  
 G G A D T D Y A D G  
 AGTGAAGACAAAGTAGTAGAAGTAGCAGAG  
 S E D K V V E V A E  
 GAGGAAGAAGTGGCTGAGGTGGAAGAAGAA  
 E E E V A E V E E E  
 GAAGCCGATGATGACGAGGACGATGAG GAT  
 E A D D D E D D E D  
 GGTGATGAGGTAGAGGAAGAGGCTGAGGAA  
 G D E V E E E A E E  
 CCCTACGAAGAAGCCACAGAGAGAACCACC  
 P Y E E A T E R T T  
 AGCATTGCCACCACCACCACCACCACCACA  
 S I A T T T T T T T  
 GAGTCTGTGGAAGAGGTGGTTCGAGTTCCT  
 E S V E E V V R V P  
 ACAACAGCAGCCAGTACCCCTGATGCCGTT  
 T T A A S T P D A V  
 GACAAGTATCTCGAGACACCTGGGGATGAG  
 D K Y L E T P G D E  
 AATGAACATGCCCATTTCCAGAAAGCCAAA  
 N E H A H F Q K A K  
 GAGAGGCTTGAGGCCAAGCACCGAGAGAGA  
 E R L E A K H R E R  
 ATGTCCCAGGTCATGAGAGAATGGGAAGAG  
 M S Q V M R E W E E  
 GCAGAACGTCAAGCAAAGAACTTGCCTAAA  
 A E R Q A K N L P K

-continued

240 GCTGATAAGAAGGCAGTTATCCAGCATTTC 1080  
 A D K K A V I Q H F  
 270 5 CAGGAGAAAGTGGAAATCTTTGGAACAGGAA 1110  
 Q E K V E S L E Q E  
 300 GCAGCCAACGAGAGACAGCAGCTGGTGGAG 1140  
 A A N E R Q Q L V E  
 330 10 ACACACATGGCCAGAGTGGAAAGCCATGCTC 1170  
 T H M A R V E A M L  
 360 AATGACCGCCGCGCCTGGCCCTGGAGAAC 1200  
 N D R R R L A L E N  
 390 15 TACATCACCGCTCTGCAGGCTGTTCTCCT 1230  
 Y I T A L Q A V P P  
 420 CGGCCTCGTCACGTGTTCAATATGCTAAAG 1260  
 R P R H V F N M L K  
 450 20 AAGTATGTCCGCGCAGAACAGAAGGACAGA 1290  
 K Y V R A E Q K D R  
 480 CAGCACACCCTAAAGCATTTCGAGCATGTG 1320  
 Q H T L K H F E H V  
 510 25 CGCATGGTGGATCCCAAGAAAGCCGCTCAG 1350  
 R M V D P K K A A Q  
 540 ATCCGGTCCCAGGTTATGACACACCTCCGT 1380  
 I R S Q V M T H L R  
 570 30 GTGATTTATGAGCGCATGAATCAGTCTCTC 1410  
 V I Y E R M N Q S L  
 600 TCCCTGCTCTACAACGTGCCTGCAGTGGCC 1440  
 S L L Y N V P A V A  
 630 35 GAGGAGATTCAGGATGAAGTTGATGAGCTG 1470  
 E E I Q D E V D E L  
 660 CTTCAGAAAGAGCAAAACTATTTCAGATGAC 1500  
 L Q K E Q N Y S D D  
 690 40 GTCTTGGCCAACATGATTAGTGAACCAAGG 1530  
 V L A N M I S E P R  
 720 ATCAGTTACGGAAACGATGCTCTCATGCCA 1560  
 I S Y G N D A L M P  
 750 TCTTTGACCGAAACGAAAACCACCGTGGAG 1590  
 S L T E T K T T V E  
 780 CTCCTTCCCGTGAATGGAGAGTTCAGCCTG 1620  
 L L P V N G E F S L  
 810 50 GACGATCTCCAGCCGTGGCATTCTTTTGGG 1650  
 D D L Q P W H S F G  
 840 GCTGACTCTGTGCCAGCCAACACAGAAAAC 1680  
 A D S V P A N T E N  
 870 GAAGTTGAGCCTGTTGATGCCCGCCCTGCT 1710  
 900 55 E V E P V D A R P A  
 930 GCCGACCGAGGACTGACCACTCGACCAGGT 1740  
 A D R G L T T R P G  
 960 60 TCTGGGTTGACAAATATCAAGACGGAGGAG 1770  
 S G L T N I K T E E  
 990 ATCTCTGAAGTGAAGATGGATGCAGAATTC 1800  
 I S E V K M D A E F  
 1020 65 CGACATGACTCAGGATATGAAGTTCATCAT 1830  
 R H D S G Y E V H H  
 1050 CAAAAATTGGTGTCTTTGCAGAAGATGTG 1860  
 Q K L V F F A E D V

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GGTTCAAACAAAGGTGCAATCATTGGACTC  
 G S N K G A I I G L  
 ATGGTGGGCGGTGTTGT CATAGCGACAGTG  
 M V G G V V I A T V  
 ATCGTCATCACCTTGGTGATGCTGAAGAAG  
 I V I T L V M L K K  
 AAACAGTACACATCCATTCATCATGGTGTG  
 K Q Y T S I H H G V  
 GTGGAGGTTGACGCCGCTGTCACCCCGAGAG  
 V E V D A A V T P E  
 GAGCGCCACCTGTCCAAGATGCAGCAGAAC  
 E R H L S K M Q Q N  
 GGCTACGAAAATCCAACCTACAAGTTCTTT  
 G Y E N P T Y K F F  
 GAGCAGATGCAGAACTAGACCCCGCCACA  
 E Q M Q N \*  
 GCAGCCTCTGAAGTTGGACAGCAAACCAT  
 TGCTTCACTACCCATCGGTGTCCATTTATA  
 GAATAATGTGGGAAGAAACAAACCCGTTTT  
 ATGATTTACTCATTATCGCCTTTTGACAGC  
 TGTGCTGTAACACAAGTAGATGCCTGAACT  
 TGAATTAATCCACACATCAGTAATGTATTC  
 TATCTCTCTTTACATTTTGGTCTCTATACT  
 ACATTATTAATGGGTTTTGTGTACTGTAAA  
 GAATTTAGCTGTATCAAACCTAGTGCATGAA  
 TAGATTCTCTCCTGATTATTTATCACATAG  
 CCCCTTAGCCAGTTGTATATTATTCTTG TG  
 GTTTGTGACCCAATTAAGTCTACTTTACA  
 TATGCTTTAAGAATCGATGGGGGATGCTTC  
 ATGTGAACGTGGGAGTTCAGCTGCTTCTCT  
 TGCCTAAGTATTCCTTTCTGATCACTATG  
 CATTTTAAAGTTAAACATTTTAAAGTATTT  
 CAGATGCTTTAGAGAGATTTTTTTTCCATG  
 ACTGCATTTTACTGTACAGATTGCTGCTTC  
 TGCTATATTTGTGATATAGGAATTAAGAGG  
 ATACACACGTTTGTTCCTCGTGCCTGTTT  
 TATGTGCACACATTAGGCATTGAGACTTCA  
 AGCTTTTCTTTTTTTGTCCACGTATCTTTG  
 GGTCTTTGATAAAGAAAAGAATCCCTGTTC  
 ATTGTAAGCACTTTTACGGGGCGGGTGGGG  
 AGGGGTGCTCTGCTGGTCTTCAATTACCAA  
 GAATTCTCCAAAACAATTTTCTGCAGGATG  
 ATTGTACAGAATCATTGCTTATGACATGAT  
 CGCTTTCTACACTGTATTACATAAATAAAT  
 TAAATAAATAACCCCGGGCAAGACTTTTC  
 TTTGAAGGATGACTACAGACATTAATAAAT

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1890 CGAAGTAATTTTGGGTGGGGAGAAGAGGCA 3030  
 1920 5 GATTCAATTTTCTTTAACCAGTCTGAAGTT 3060  
 TCATTTATGATACAAAAGAAGATGAAAATG 3090  
 1950 GAAGTGGCAATATAAGGGGATGAGGAAGGC 3120  
 1980 10 ATGCCTGGACAAACCCTTCTTTTAAGATGT 3150  
 2010 GTCTTCAATTTGTATAAAATGGTGTTTTCA 3180  
 TGTAATAAATACATTCTTGGAGGAGC-poly(A)tail  
 2040 15 and functional equivalents thereof.  
 2070 2. A deoxyribonucleic acid according to claim 1 of  
 the sequence:  
 2100 ATGCTGCCCGGTTTGGCACTGCTCCTGCTG 30  
 20 M L P G L A L L L L  
 2130 GCCGCCTGGACGGCTCGGGCGCTGGAGGTA 60  
 A A W T A R A L E V  
 2160 CCCACTGATGGTAATGCTGGCCTGCTGGCT 90  
 2190 25 P T D G N A G L L A  
 2220 GAACCCAGATTGCCATGTTCTGTGGCAGA 120  
 E P Q I A M F C G R  
 2250 CTGAACATGCACATGAATGTCCAGAATGGG 150  
 2280 30 L N M H M N V Q N G  
 2310 AAGTGGGATTCAGATCCATCAGGGACCAAA 180  
 K W D S D P S G T K  
 2340 ACCTGCATTGATACCAAGGAAGGCATCCTG 210  
 2370 35 T C I D T K E G I L  
 2400 CAGTATTGCCAAGAAGTCTACCCTGAACTG 240  
 Q Y C Q E V Y P E L  
 2430 CAGATACCAATGTGGTAGAAGCCAACCAA 270  
 2460 40 Q I T N V V E A N Q  
 2490 CCAGTGACCATCCAGAAGTGGTGAAGCGG 300  
 P V T I Q N W C K R  
 2520 GGCCGCAAGCAGTGCAAGACCCATCCCCAC 330  
 2550 45 G R K Q C K T H P H  
 2580 TTTGTGATTCCCTACCGCTGCTTAGTTGGT 360  
 F V I P Y R C L V G  
 2610 GAGTTTGTAAAGTATGATGCCCTTCTCGTTCT 390  
 2640 E F V S D A L L V P  
 2670 50 GACAAGTGCAAATTCTTACACCAGGAGAGG 420  
 D K C K F L H Q E R  
 2700 ATGGATGTTTGCGAAACTCATCTTCACTGG 450  
 2730 M D V C E T H L H W  
 2760 55 CACACCGTCGCCAAAGAGACATGCAGTGAG 480  
 2790 H T V A K E T C S E  
 2820 AAGAGTACCAACTTGCATGACTACGGCATG 510  
 K S T N L H D Y G M  
 2850 60 TTGCTGCCCTGCGGAATTGACAAGTTCCGA 540  
 2880 L L P C G I D K F R  
 2910 GGGGTAGAGTTTGTGTGTTGCCCACTG GCT 570  
 G V E F V C C P L A  
 2940 65 GAAGAAAGTGACAATGTGGATTCTGCTGAT 600  
 2970 E E S D N V D S A D  
 3000 GCGGAGGAGGATGACTCGGATGTCTGGTGG 630  
 A E E D D S D V W W



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GGCGGAGCAGACACAGACTATGCAGATGGG 660  
 G G A D T D Y A D G

AGTGAAGACAAAGTAGTAGAAGTAGCAGAG 690  
 S E D K V V E V A E

GAGGAAGAAGTGGCTGAGGTGGAAGAAGAA 720  
 E E E V A E V E E E

GAAGCCGATGATGACGAGGACGATGAGGAT 750  
 E A D D D E D D E D

GGTGATGAGGTAGAGGAAGAGGCTGAGGAA 780  
 G D E V E E E A E E

CCCTACGAAGAAGCCACAGAGAGAACCACC 810  
 P Y E E A T E R T T

AGCATTGCCACCACCACCACCACCACCACA 840  
 S I A T T T T T T T

GAGTCTGTGGAAGAGGTGGTTCGAGTTCCT 870  
 E S V E E V V R V P

ACAACAGCAGCCAGTACCCCTGATGCCGTT 900  
 T T A A S T P D A V

GACAAGTATCTCGAGACACCTGGGGATGAG 930  
 D K Y L E T P G D E

AATGAACATGCCATTTCCAGAAAGCCAAA 960  
 N E H A H F Q K A K

GAGAGGCTTGAGGCCAAGCACCGAGAGAGA 990  
 E R L E A K H R E R

ATGTCCCAGGTCATGAGAGAATGGGAAGAG 1020  
 M S Q V M R E W E E

GCAGAACGTCAAGCAAAGAACTTGCCTAAA 1050  
 A E R Q A K N L P K

GCTGATAAGAAGGCAGTTATCCAGCATTTC 1080  
 A D K K A V I Q H F

CAGGAGAAAGTGAATCTTTGGAACAGGAA 1110  
 Q E K V E S L E Q E

GCAGCCAACGAGAGACAGCAGCTGGTGGAG 1140  
 A A N E R Q Q L V E

ACACACATGGCCAGAGTGAAGCCATGCTC 1170  
 T H M A R V E A M L

AATGACCGCCGCCGCTGGCCCTGGAGAAC 1200  
 N D R R R L A L E N

TACATCACCGCTCTGCAGGCTGTTCTCCT 1230  
 Y I T A L Q A V P P

CGGCCTCGTCACGTGTTCAATATGCTAAAG 1260  
 R P R H V F N M L K

AAGTATGTCCGCGCAGAACAGAAGGACAGA 1290  
 K Y V R A E Q K D R

CAGCACACCCTAAAGCATTTCGAGCATGTG 1320  
 Q H T L K H F E H V

CGCATGGTGGATCCCAAGAAAGCCGCTCAG 1350  
 R M V D P K K A A Q

ATCCGGTCCCAGGTTATGACACACCTCCGT 1380  
 I R S Q V M T H L R

GTGATTTATGAGCGCATGAATCAGTCTCTC 1410  
 V I Y E R M N Q S L

TCCCTGCTCTACAACGTGCCTGCAGTGGCC 1440  
 S L L Y N V P A V A

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GAGGAGATTCAGGATGAAGTTGATGAGCTG 1470  
 E E I Q D E V D E L

5 CTTCAGAAAGAGCAAACTATTCAGATGAC 1500  
 L Q K E Q N Y S D D

GTCTTGGCCAACATGATTAGTGAACCAAGG 1530  
 V L A N M I S E P R

10 ATCAGTTACGGAAACGATGCTCTCATGCCA 1560  
 I S Y G N D A L M P

TCTTTGACCGAAACGAAAACCACCGTGGAG 1590  
 S L T E T K T T V E

15 CTCCTTCCCGTGAATGGAGAGTTCAGCCTG 1620  
 L L P V N G E F S L

GACGATCTCCAGCCGTGGCATTCTTTTGGG 1650  
 D D L Q P W H S F G

GCTGACTCTGTGCCAGCCAACACAGAAAAC 1680  
 20 A D S V P A N T E N

GAAGTTGAGCCTGTTGATGCCCGCCCTGCT 1710  
 E V E P V D A R P A

GCCGACCGAGGACTGACCACTCGACCAGGT 1740  
 25 A D R G L T T R P G

TCTGGGTTGACAAATATCAAGACGGAGGAG 1770  
 S G L T N I K T E E

ATCTCTGAAGTGAAGATGGATGCAGAATTC 1800  
 30 I S E V K M D A E F

CGACATGACTCAGGATATGAAGTTCATCAT 1830  
 R H D S G Y E V H H

CAAAAATTGGTGTCTTTGCAGAAGATGTG 1860  
 35 Q K L V F F A E D V

GGTTCAAACAAAGGTGCAATCATTGGACTC 1890  
 G S N K G A I I G L

ATGGTGGGCGGTGTTGTCATAGCGACAGTG 1920  
 40 M V G G V V I A T V

ATCGTCATCACCTTGGTGTGCTGAAGAAG 1950  
 I V I T L V M L K K

AAACAGTACACATCCATTCATCATGGTGTG 1980  
 45 K Q Y T S I H H G V

GTGGAGGTTGACGCCGCTGTCACCCCAGAG 2010  
 V E V D A A V T P E

GAGCGCCACCTGTCCAAGATGCAGCAGAAC 2040  
 E R H L S K M Q Q N

50 GGCTACGAAAATCCAACCTACAAGTTCTTT 2070  
 G Y E N P T Y K F F

GAGCAGATGCAGAACTAGA  
 E Q M Q N \*

55 and functional equivalents thereof.

3. A deoxyribonucleic acid fragment according to claim 1 of the sequence:

60 GGGGTAGAGTTTGTGTGTTGCCCACTGGCT 570  
 G V E F V C C P L A

GAAGAAAGTGACAATGTGGATTCTGCTGAT 600  
 E E S D N V D S A D

65 GCGGAGGAGGATGACTCGGATGTCTGGTGG 630  
 A E E D D S D V W W

GGCGGAGCAGACACAGACTATGCAGATGGG 660  
 G G A D T D Y A D G



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AGTGAAGACAAAGTAGTAGAAGTAGCAGAG  
S E D K V V E V A E

GAGGAAGAAGTGGCTGAGGTGGAAGAAGAA  
E E E V A E V E E E

GAAGCCGATGATGACGAGGACGATGAGGAT  
E A D D D E D D E D

GGTGATGAGGTAGAGGAAGAGGCTGAGGAA  
G D E V E E E A E E

-continued

690 CCCTACGAAGAAGCCACAGAGAGAACCACC 810  
P Y E E A T E R T T

5 AGCATTGCCACCACCACCACCACCACACA 840  
S I A T T T T T T T

720 GAGTCTGTGGAAGAGGTGGTTCGAGTTCCT 870  
E S V E E V V R V P

750 10 ACAACAGCAGCCAGTACCCCTGATGCCGTT 900  
T T A A S T P D A V

780 and functional equivalents thereof.  
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